Neuroscience 196 (2011) 1–15

MOLECULAR ADAPTATIONS OF APOPTOTIC PATHWAYS AND SIGNALING PARTNERS IN THE CEREBRAL CORTEX OF HUMAN COCAINE ADDICTS AND COCAINE-TREATED RATS

M. ÁLVARO-BARTOLOMÉ,[a] R. LA HARPE,[b] L. F. CALLADO,[c] J. J. MEANA[c] AND J. A. GARCÍA-SEVILLA[a]*

[a]*Laboratorio de Neurofarmacología, Institut Universitari d'Investigació en Ciències de la Salut (IUNICS), Universitat de les Illes Balears (UIB) and Red Temática de Investigación Cooperativa en Salud (RETICS-RTA), Cra. Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain*

[b]*Centre Universitaire Romand de Médecine Légale–Site Genève, Faculté de Médecine, Université de Genève, Avenue de Champel 9, CH-1211 Genève 4, Switzerland*

[c]*Departamento de Farmacología, Universidad del País Vasco (UPV/EHU) and Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), E-48109 Leioa, Bizkaia, Spain*

Abstract—Cocaine induces apoptotic effects in cultured cells and in the developing brain, but the aberrant activation of cell death in the adult brain remains inconclusive, especially in humans. This postmortem human brain study examined the status of canonical apoptotic pathways, signaling partners, and the cleavage of poly(ADP-ribose) polymerase-1 (PARP-1), a sensor of DNA damage, in prefrontal cortex (PFC) of a small but well-characterized cohort of cocaine abusers (n=10). For comparison, the chosen targets were also quantified in the cerebral cortex of cocaine-treated rats. In the PFC of cocaine abusers, FS7-associated cell surface antigen (Fas) receptor aggregates and Fas-associated death domain (FADD) adaptor were reduced (−26% and −66%, respectively) as well as the content of mitochondrial cytochrome c (−61%). In the same brain samples of cocaine abusers, the proteolytic cleavage of PARP-1 was increased (+39%). Nuclear PARP-1 degradation, possibly a consequence of increased mitochondrial oxidative stress, involved the activation of apoptosis-inducing factor (AIF) and not that of caspase-3. In the PFC of cocaine abusers, several signaling molecules associated with cocaine/dopamine and/or apoptotic pathways were not significantly altered, with the exception of anti-apoptotic truncated DARPP-32 (t-DARPP), a truncated isoform of dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), whose content was decreased (−28%). Chronic exposure to cocaine in rats, including withdrawal for 3 days, did not alter Fas–FADD receptor complex, cytochrome c, caspase-3/fragments, AIF, PARP-1 cleavage, and associated signaling in the cerebral cortex. Chronic cocaine and abstinence, however, increased the content of t-DARPP (+39% and +47%) in rat brain cortex. The major findings indicate that cocaine addiction in humans is not associated with abnormal activation of extrinsic and intrinsic apoptotic pathways in PFC. The down-regulation of Fas–FADD receptor complex and cytochrome c could reflect the induction of contraregulatory adaptations or non-apoptotic (neuroplastic) actions induced by the psychostimulant. The enhanced degradation of nuclear PARP-1, a hallmark of apoptosis, indicates the possibility of aberrant cell death.

Key words: cocaine addiction, postmortem human brain, rat brain, Fas–FADD complex, PARP-1 cleavage, apoptotic signaling.

Cocaine addiction is a persistent, relapsing, and behavioral disorder that constitutes a major medical problem in modern societies (O'Brien and Anthony, 2005). Cocaine is a powerful psychostimulant affecting the reuptake of dopamine by nerve terminals through inhibition of its transporter (DAT), which in the short-term leads to increased dopamine neurotransmission in the brain (Ritz et al., 1987; Giros et al., 1996). Cocaine also blocks noradrenaline and serotonin transporters and potentiates neurotransmitter actions in non-dopaminergic brain regions (Bennett et al., 1995). Moreover, repeated treatment with cocaine has been shown to sensitize noradrenergic and serotonergic neurons through non-dopaminergic mechanisms (Lanteri et al., 2008). The toxicity of cocaine involves a plethora of cardiovascular complications (e.g. myocardial infarction; Isner et al., 1986) and cerebrovascular disorders (e.g. ischemic stroke; Toossi et al., 2010). In the long-term, cocaine addiction is associated with aberrant neuroplasticity (Thomas et al., 2008) and several dysfunctions (Büttner, 2011) in the brain, which could be partly related to the activation of cell death mechanisms.

Apoptosis is a cell self-destructive process that requires receptor transmission of apoptotic signals and the formation of a death-inducing signaling complex (DISC) [e.g. FS7-associated cell surface antigen (Fas) receptor–Fas-associated death domain (FADD) adaptor, initiator caspase-8, and FADD-like interleukin-1β-converting enzyme-inhibitory protein ($FLIP_L$); Algeciras-Schimnich et al., 2002; Salvesen and Riedl, 2009; Hymowitz and Dixit,

*Corresponding author. Tel: +34-971-173148; fax: +34-971-173184.
E-mail address: jesus.garcia-sevilla@uib.es (J. A. García-Sevilla).

Abbreviations: AIF, apoptosis-inducing factor; Akt/PKB, v-akt murine thymoma viral oncogene homologue/protein kinase B; Bax, Bcl-2 associated X protein; Bcl-2, B-cell leukemia 2; CK1α, casein kinase 1α; DARPP-32, dopamine- and cAMP-regulated phosphoprotein of 32 kDa; DAT, dopamine transporter; DISC, death-inducing signaling complex; ECL, enhanced chemiluminescence; FADD, Fas-associated protein with death domain; Fas, FS7-associated cell surface antigen or tumor necrosis factor receptor superfamily 6 (TNFRSF6); FLIP-L, FADD-like interleukin-1β-converting enzyme-inhibitory protein; JNK, c-jun NH2-terminal protein kinase or stress-activated protein kinase (SAPK); MAPK, mitogen-activated protein kinase; p, phosphorylated; PAR, poly(ADP-ribose) polymers; PAR-4, nuclear protein prostate apoptosis response; PARP-1, poly(ADP-ribose) polymerase-1; PEA-15, phosphoprotein enriched in astrocytes of 15 kDa; PFC/BA9, prefrontal cortex/Brodmann's area 9; PKA, protein kinase A; PMD, postmortem delay; SDS, sodium dodecyl sulfate; t-DARPP, truncated DARPP-32.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.08.074

2010; Peter, 2011]. Apoptosis also requires the involvement of pro-apoptotic factors inside the cell such as the activation of mitochondrial proteins including the release of cytochrome c (Galluzzi et al., 2009), which have important roles in detecting and amplifying the apoptotic signals of death receptors. It is well established that apoptosis in brain tissue involves both the extrinsic and the intrinsic/mitochondrial pathways (Lossi and Merighi, 2003), which converge to activate executioner molecules such as caspases-3/7 and apoptosis-inducing factor (AIF) with the final cleavage of vital substrates and cell death (Kumar, 2007).

Acute and/or chronic exposure to cocaine was reported to induce apoptosis in fetal rat myocardial cells (Xiao et al., 2000), to increase the expression of pro-apoptotic genes in developing cerebral cells (Novikova et al., 2005; Lee et al., 2009), and to upregulate the content of some pro-apoptotic mitochondrial proteins in cultured cells and brain tissue (Cunha-Oliveira et al., 2008, 2010). For example, fetuses (E18) of chronically cocaine-treated pregnant mice showed an increased expression of apoptosis-related genes including those for death receptors, adaptor proteins, and effector caspases in frontal and occipital regions of the cerebral wall (Novikova et al., 2005), which may compromise neuronal survival in the developing brain. Similarly, exposure to cocaine of primary human fetal cerebral cortex cells at 20 weeks of gestation resulted in upregulation of pro-apoptotic genes in glial cells (Lee et al., 2009). Prenatal cocaine has also been reported to induce changes in brain function in children (Ackerman et al., 2010). However, whether or not cocaine and/or metabolites can induce neurotoxicity and apoptosis in the adult mammalian brain remains inconclusive (Dietrich et al., 2005; García-Fuster et al., 2009). Moreover, the possible induction of aberrant apoptosis in brains of human cocaine abusers has not yet been explored.

This study tested the hypothesis that cocaine addiction results in abnormal activation of canonical apoptotic pathways in the human brain. In line with previous postmortem brain studies on the regulation of death pathways in opiate addicts (García-Fuster et al., 2008b; Ramos-Miguel et al., 2009), the main components of the extrinsic and intrinsic apoptotic pathways were quantified in the prefrontal cortex (PFC) of a selected cohort of cocaine abusers. The apoptotic targets included Fas receptor, FADD and p-Ser194 FADD adaptor, caspase-8/fragments, $FLIP_L$, Bax, Bcl-2, cytochrome c, caspase-3/fragments, AIF, and the cleavage of poly(ADP-ribose) polymerase-1 (PARP-1), a sensor of DNA damage (García-Fuster et al., 2008b). Furthermore, various dopaminergic signaling molecules involved in the molecular mechanisms of cocaine addiction, cell death by apoptosis, and/or neuroplasticity were also investigated. The dopamine targets included DAT (Ritz et al., 1987), dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32 and p-Thr34 DARPP-32 mediated by protein kinase A, PKA; Nairn et al., 2004), and truncated DARPP (t-DARPP), a 30-kDa isoform of DARPP-32 with striking anti-apoptotic actions (El-Rifai et al., 2002). Brain imaging studies with radiotracers have revealed that cocaine dependence in humans is associated with decreased D_2 receptor binding potential, a composite of both receptor density and affinity, and a deficit in dopamine neurotransmission (Martinez and Narendran, 2010). Finally, the status of relevant kinases and dependent factors involved in apoptotic signaling were also assessed. These targets included pro-apoptotic c-Jun NH_2-terminal kinases (JNK1/2; Dhanasekaran and Reddy, 2008), anti-apoptotic kinase Akt (Akt1; Song et al., 2005), and phosphoprotein enriched in astrocytes of 15 kDa (PEA-15; Araujo et al., 1993), an anti-apoptotic factor when phosphorylated at Ser116 by Akt1 (Trencia et al., 2003). For comparison, the canonical apoptotic pathways and associated partners were also quantified in the cerebral cortex of rats after acute and chronic treatments with cocaine and during abstinence to the psychostimulant.

The major findings indicate that human cocaine addiction is associated with downregulation of Fas–FADD receptor complex and mitochondrial cytochrome c, as well as with an increased degradation of nuclear PARP-1 in the prefrontal cortex.

EXPERIMENTAL PROCEDURES

Postmortem brain samples of cocaine abusers and healthy controls

Specimens of PFC, middle frontal gyrus, Brodmann's area 9 (BA9) from cocaine abusers and healthy controls were obtained at autopsies performed in the Centre Universitaire Romand de Médecine Légale–Site Genève, University of Geneva, Switzerland, following the established legal and ethical procedures. The bodies had been stored in a refrigerator at 4 °C until autopsy. The right part of the PFC/BA9 was selected for examination to keep in line with previous postmortem studies on the molecular mechanisms of opiate addiction in humans (Escribá et al., 1994; Ozaita et al., 1998; Meana et al., 2000; Ferrer-Alcón et al., 2003, 2004; García-Fuster et al., 2008b; Ramos-Miguel et al., 2009). The PFC (Fuster, 2001) is a relevant brain region in cocaine addiction because of its role in cognitive control over drug intake and its association with the mesocorticolimbic dopaminergic system that mediates the rewarding and addictive properties of most drugs of abuse (Koob and Volkow, 2010; Bradberry, 2011). In chronic cocaine users, cocaine and metabolites were shown to be similarly distributed in the PFC, which expresses a low DAT content, and specific dopamine-rich areas with high DAT contents (Kalasinsky et al., 2000). Samples were dissected from ~5-g pieces of PFC/BA9, only gray matter, and stored at −80 °C. The experiments were performed in the Laboratorio de Neurofarmacología–IUNICS, University of the Balearic Islands (UIB, Spain), after formal approval from the local Ethical Committee of Clinical Investigation (CEIC) and in accordance with the guidelines of UIB.

Brain samples were taken from 10 well-characterized cocaine abusers and 10 healthy matched controls (Table 1). A limited number of "pure" cocaine abusers (collecting period: 1999–2008) could be included in the study because of the very short availability of subjects who had abuse of intranasal, smoked/inhaled, or i.v. cocaine only. Mixed cocaine/opiate addicts were excluded from the study, even if no history of opiate abuse was documented (heroin or methadone were incidental to the cause of death). The combination of heroin and cocaine has been shown to potentiate neurotoxicity (caspase-3-dependent apoptosis) in cortical neurons in culture (Cunha-Oliveira et al., 2010). Cocaine abusers with positive blood toxicology for amphetamine and methamphetamine derivatives, cannabinoids, antidepressants, and/or antipsychotics